Avian Influenza

Disease Monograph Series – 04

Virus | *Orthomyxoviridae* | Poultry

IDRC | Bartay

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Acronyms

AGID	Agar Gel Immunodiffusion assay
AI	Avian influenza
ASEAN	Association of South East Asian Nations
AU-IBAR	African Union InterAfrican Bureau for Animal Resources
AU	African Union
DIVA	Differentiating infected from vaccinated animals (strategy)
DVE	Duck virus enteritis (duck plague)
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
H	Hemagglutinin subtype (e.g. H5)
HA	Hemagglutination antigen
HI	Hemagglutination inhibition
IB	Infectious bronchitis
IBD	Infectious bursal disease
HPAI	Highly pathogenic avian influenza
IVPI	Intravenous pathogenicity index
LBM	Live bird market
LPAI	Low pathogenic avian influenza

N	Neuraminidase subtype (e.g. N1)
NA	Neuraminidase antigen
NDV	Newcastle disease virus
NI	Neuraminidase inhibition
OIE	World Organization for Animal Health
RT-PCR	Reverse transcription polymerase chain reaction
SAARC	South Asia Association for Regional Cooperation
SEPRL	Southeast Poultry Research Laboratory
TADs	Transboundary animal diseases
USA	United States of America
VI	Virus isolation
WAHID	Interface for the World Animal Health Information System (WAHIS)
WAHIS	World Animal Health Information System (database)
WHO	World Health Organization

Executive Summary

Avian influenza (AI) belongs to a highly mutable, reportable group of Type A influenza viruses, some of which are zoonotic. AI viruses in poultry are classified as either low pathogenic (LPAI) or highly pathogenic (HPAI) based on the clinical signs and mortality with a chicken host challenge model. Three major waves occurred in Europe between 1876 and 1931 following the initial identification of the agent of "fowl plague" near the end of the 19th Century. Over a span of 136 years between 1876 and 2012, there were at least 97 epizootics of AI recorded globally [8]. AI viruses in poultry are classified as either low pathogenic (LPAI) or highly pathogenic (HPAI) based on the clinical signs and mortality with a chicken host challenge model.

The epidemiology of avian influenza viruses can be understood following the principle of the epidemiological triad, including agent, host and environmental characteristics. Waterfowl, both domesticated and wild play an important role as the sometimes "silent" reservoir, particularly in Asia. Waterfowl promote endemicity of the virus in this region, unlike Africa. H5N1 Eurasian subtype is only the second recorded AI virus to kill its waterfowl reservoir hosts. H9N2 subtype is also a zoonosis and warrants further scrutiny and attention.

Detection of AI through surveillance and measuring the socio-economic impact remain challenging, despite a total 844 human cases and 449 deaths due to H5N1 resulting in a case fatality rate of 53% since 2003. Unprecedented prior to 1997, H5N1, H7N9 and H9N2 have been most commonly associated with human morbidity and mortality. The case fatality rate greatly exceeds that of annual season influenza in humans. Prevention and control of AI is deemed to serve the Global Public Good, however, veterinary capacity in many developing and developed countries remains limited [27].

The Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) guidance for the prevention and control of HPAI H5N1 include humane culling of affected stock, movement control, disease surveillance, biosecurity, and vaccination. Vaccination is only one tool which can used, however by itself cannot eliminate the virus. Vaccine also places further selection pressure for additional drift and adaptation. Both technical and non-technical factors should be considered when applying vaccination judiciously. Inactivated, recombinant and vectored vaccines and related research are reviewed.

Gaps in knowledge or capacity impacting strategic planning and effective implementation

The following gaps are highlighted in relation to vaccine development and sustainable field implementation for avian influenza:

1. The extent of AI vaccine used which does not conform to the OIE standards for quality of vaccines produced;

2. The need for environmental assessments of replicating recombinant vector vaccines;
3. The need to develop non-replicating recombinant vector vaccines in order to speed up vaccine licensing and registration;
4. Standardized laboratory diagnosis of AI using approved reagents, including primers;
5. Lack of active and passive surveillance systems;
6. Effective programmatic implementation of Differentiating infected from vaccinated animals (DIVA) strategy in more developed and less developed countries remains a challenge. Challenges are related to policy uncertainties as well as laboratory, field epidemiology and operational capacity limitations.
7. China, Viet Nam and Indonesia have ceased to vaccinate smallholder poultry. Sustainable models for successful community engagement to improve logistics and delivery of AI vaccine for smallholders.

Options and Strategies for Vaccination

Vaccination induces protection to poultry mediated through humoral, cellular, and innate immunity. The immunity that results from vaccination does not provide sterilizing immunity, but it can reduce clinical signs and mortality and reduce virus shedding, resulting in reduced contact transmission among poultry. Inactivated vaccines are most commonly used against H5N1 virus, including traditional and reverse genetics vaccines; however, a number of live-vector vaccines are being increasingly used [29][30]. Key strategic considerations include [7][33]:

1. Vaccine quality, selection, non-replicating and regular matching with circulating field strains;
2. Vaccination strategy – mass/targeted; prophylactic use in breeders/rare breeds; emergency use to protect poultry, farmers and public health; selection of target group;
3. Implementation of vaccination program – public-private partnerships, logistics, procurement, storage, cold chain delivery system, quality control (records)
4. Post-vaccination seromonitoring and evaluation of the vaccination program disease surveillance, and operational research studies to assess impact.
5. Use of multivalent vaccines may be a scientifically valid approach to take when it is possible. The reality in the field is that many economically significant poultry diseases are neither reported nor assessed quantitatively. Vaccine cost as well as farmer and government perception of risk must be carefully assessed in order to prioritize resources for sustainable, self-directed vaccination programs. Needs assessments of the primary stakeholders should first be conducted prior to embarking on prevention

and control through vaccination. In this way, vaccination, biosecurity training and surveillance systems incorporated specific measures for priority diseases as well as AI. The use of NDV-AI vaccines can be considered depending on the results of an epidemiological risk assessment.

Short-, medium- and long-term strategies are proposed to inform the future Livestock Vaccination Innovation Fund as it moves ahead to address this important disease agent from global to local levels.

Short-term Solutions: Inactivated AI vaccines are still effective, produce good titers and can be applied to short-lived poultry. One dose might work for short-lived birds. Proper delivery of vaccine to smallholder with community engagement is a key gap to overcome logistical challenges for the safe and effective delivery of vaccine.

Medium-term Solutions: Some replicating and especially non-replicating recombinant vector vaccines hold promise to minimize barriers to wider and more timely legal licensure, registration and use, particularly for bivalent NDV-AI combinations for chickens and other gallinaceous poultry.

Long-term Solutions: There are two main needs: 1) development of a greater variety of non-replicating AI vector vaccine models, which can be scaled up rapidly, and are environmentally safe; and 2) development of a safe, non-replicating effective bivalent Duck virus enteritis-avian influenza (DVE-AI) vaccine for ducks, particularly in Asia.

Clinical disease overview

Etiology

Influenza viruses belong to the family *Orthomyxoviridae* that cause febrile catarrhal respiratory disease of the upper respiratory tract in humans, horses, dogs, domestic pigs, avian species, mink and a variety of marine mammals including seals. Orthomyxoviruses are classified as either Types A, B or C with Type A being most pathogenic historically.

Avian influenza is a highly mutable Type A influenza virus (commonly referred to as influenza A). The lipid envelope makes it unstable and relatively susceptible to environmental destruction from ultraviolet light, chemicals, desiccation, etc.

The hemagglutinin (HA) antigen is a protein that provides the mechanism for host cell entry while the neuraminidase (NA) protein permits exit of newly replicated virions from the host cell. Proteolytic cleavage of HA into HA1 and HA2 is required for fusion of the virion with the host cell membrane and thus, for infectivity. The HA protein is the major antigen which stimulates the host immune response and protective antibodies to protect against clinical signs and death. There are at least 16 H strains and 9 N strains, which can result in 144 possible combinations and permutations and this forms the basis for serological classification based on the hemagglutinin inhibition (HI) and neuraminidase inhibition (NI) tests.

Virus structure

The virion is composed of a helical nucelocapsid containing 8 segments of single-stranded RNA which codes for 10 proteins eight (8) of which are structural (HA, NA, NP, M1, M2, PB1, PB2 and PA) and two of which are non-structural provided by the host (NS1, NS2). Virus replication of this single stranded RNA virus is highly variable, resulting in a constantly evolving and highly mutable virus [1].

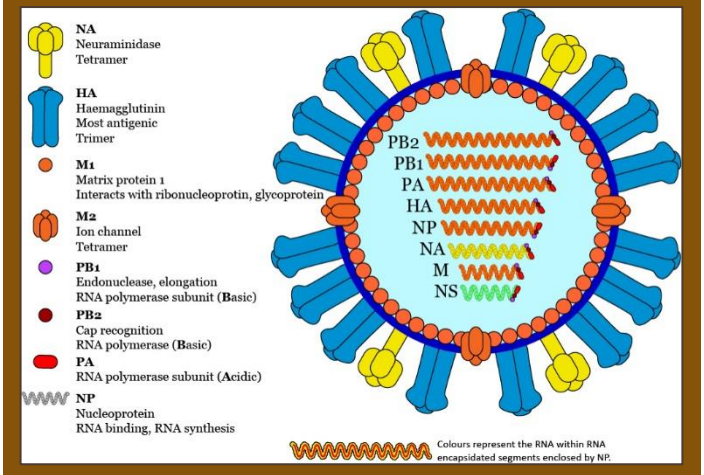

Figure 1: Schematic structural components of am influenza virion

Virus nomenclature is based on subtype, influenza type, species source, location found, strain number and year of isolation e.g. H5N2 A/chicken/Pennsylvania, 1370/83.

Antigenic variation of the HA and NA surface glycoproteins occurs at a high frequency through minor "drift" changes and may be associated with immune pressure through vaccination of poultry. Major antigenic "shift" in the HA and NA coding proteins is the result of genetic re-assortment between gene segments of two different influenza virus strains (subtypes) in host cells commonly occurs, particularly in live bird markets (LBM) where mixing of domestic waterfowl and poultry is an ongoing process of the poultry value chains in the majority of developed and developing countries.

AI viruses in poultry are classified as either low pathogenic (LPAI) or highly pathogenic (HPAI) based on the clinical signs and mortality with a chicken host challenge model. The criteria for HPAI specified by the World Organization for Animal Health (OIE) are as follows [2]:

1. *One of the two following methods to determine pathogenicity in chickens is used. A high pathogenicity influenza A virus is:*
    - *i) any influenza A virus that is lethal for six, seven or eight of eight 4- to 8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid or*
    - *ii) any influenza A virus that has an intravenous pathogenicity index (IVPI) greater than 1.2.*
2. *For all H5 and H7 viruses of low pathogenicity in chickens, the amino acid sequence of the connecting peptide of the hemagglutinin must be determined. If the sequence is similar to that observed for other HPAI isolates, the isolate being tested will be considered to be HPAI* [4].

The longer LPAI viruses are permitted to circulate in a poultry population, the higher the potential to adapt to the new host and to become more pathogenic in poultry. Historically, LPAI H5 and H7 subtypes are most likely to evolve into become highly pathogenic [4].

Avian influenza viruses preferentially bind to upper respiratory epithelium on sialoligosaccharide α2,3 receptors, human influenza viruses bind at sialoligosaccharide α2,6 receptors and pigs (swine) possess both receptor types. For this reason, pigs are considered a "mixing vessel" between avian and human species. However since 1996 and 2013, respectively the evolution of H5N1 and H7N9 subtypes directly from avian species to humans is now widely acknowledged.

Epidemiology

HPAI viruses are reported more frequently globally than LPAI, as they are reportable by the OIE member countries. Figures 2 and 3 demonstrate the respective global spatial and temporal distributions of general avian influenza and H5N1 subtype disease events reported either actively or passively:

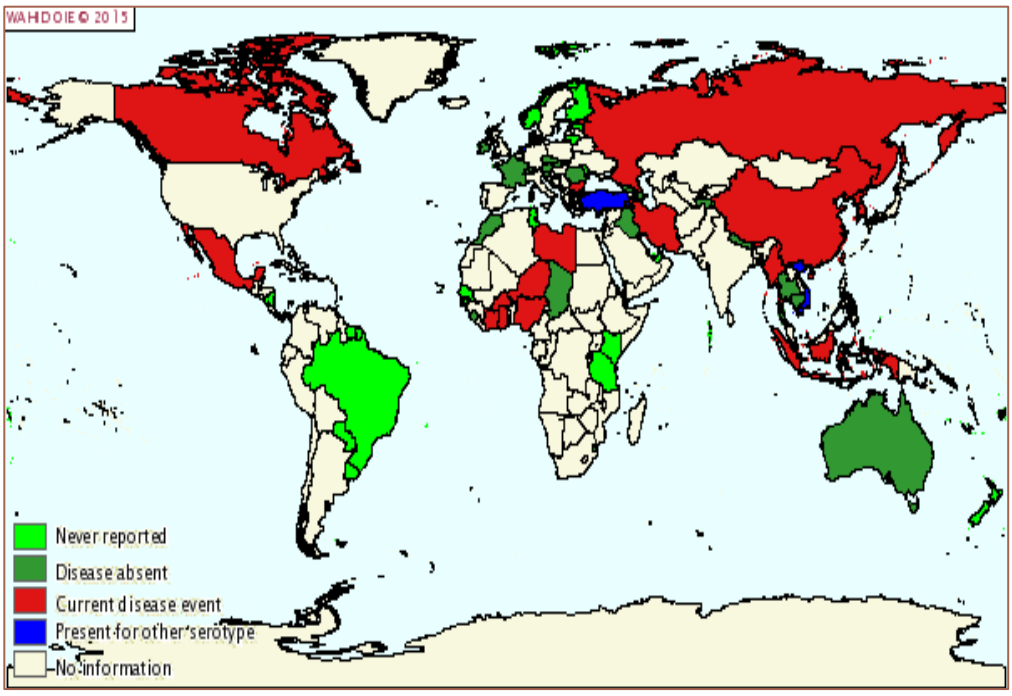

Figure 2: Spatial Distribution of Avian Influenza Viruses Reported to OIE in 2015 [6]

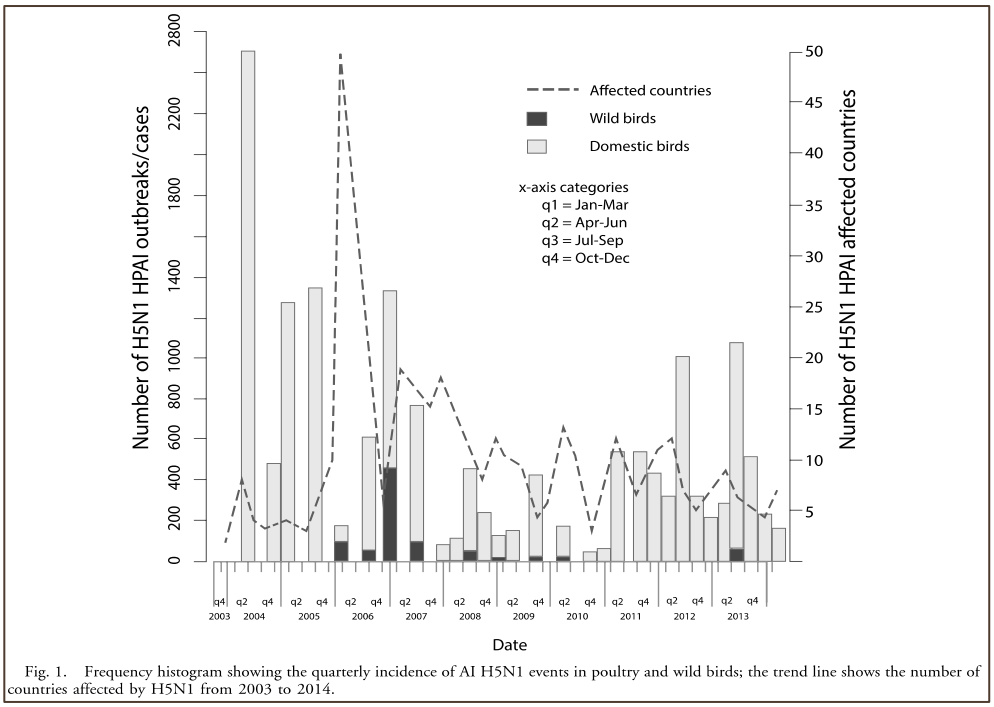

Fig. 1. Frequency histogram showing the quarterly incidence of AI H5N1 events in poultry and wild birds; the trend line shows the number of countries affected by H5N1 from 2003 to 2014.

Figure 3: Temporal Distribution of H5N1 HPAI Viruses, 2003-2013 [7]

Following the initial identification of the agent of "fowl plague" near the end of the 19th Century three major waves of AI in poultry occurred in Europe between 1876 and 1931. Over a span of 136 years between 1876 and 2012, there were at least 97 epizootics of AI recorded globally [8]. Within this historical context, the scale and scope of the recent highly pathogenic avian influenza (HPAI) H5N1 epizootic is one of the largest recorded. Since 2003, H5N1 subtype has been reported in poultry or wild birds from 50 countries as noted in Figure 3 [8]. Figure 4 presents a predictive map to assess future risk of avian influenza viruses in globally, including Africa and Asia [8]:

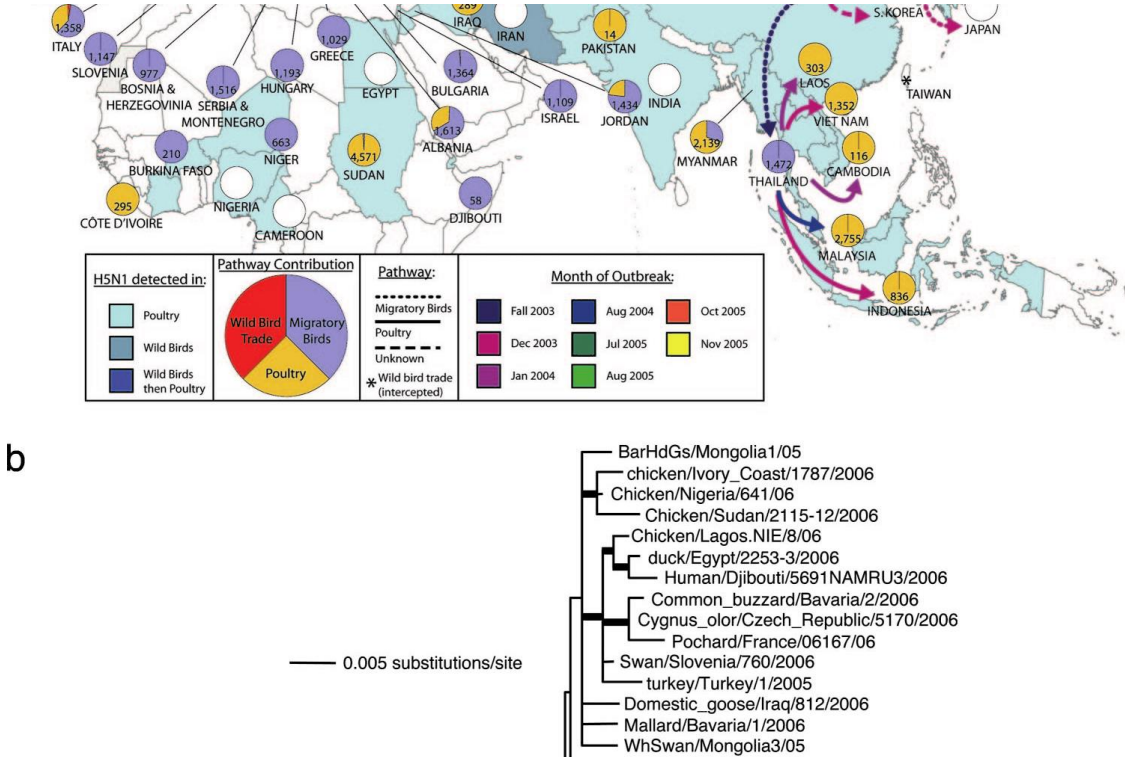

Figure 4: Spread of H5N1 in Asia, Europe, and Africa. Pie charts show the total number of infectious bird days (number of infected birds days shedding virus) and fraction from each pathway for birds moving between previous H5N1 outbreak countries and the focal country [8].

The epidemiology of avian influenza viruses is examined in terms of the epidemiological triad, including agent, host and environmental characteristics.

Agent Factors

Avian influenza viruses are highly mutable and relatively susceptible to environmental degradation [1]. Several avian influenza viruses are considered zoonoses, causing mortality and morbidity in humans including H5N1 (globally), H7N7 (Netherlands), H7N9 and H9N2 (China and Hong Kong) [5]. Importantly, H7N9 is the first LPAI virus reported to cause mortality in humans. Inter-species spillover and transmission has been documented between avian species, swine and humans. This linkage is based on molecular receptor binding affinity. To improve resolution of disease risk at the human-animal interface, H5N1 HPAI viruses have been sub-classified according to Clades, or a group of AI viruses that share a common ancestor. There are at least 10 Clade groups currently identified by the OIE and FAO Network of expertise on animal influenza (OFFLU) [9].

Both antigenic shift and drift are important mechanisms for virus evolution. The presence of co-circulating reasserting subtypes among dense poultry populations adds pressure for antigenic shift. LPAI H9N2 has circulated for decades in countries from North Africa to the East China coastline and contributed its 6 internal genes to H5N1, H5N6, H5N8, H7N9 and H10N8. During late 2014 and early 2015, H5N2 Clade 2.3.4.6 from Eastern China has been identified as the likely precursor of H5N8 reported in China, Japan, North Korea and Europe as well as H5N6 reported in China, Lao PDR and Viet Nam. Intrinsic subtype specific antigenic drift is associated with the frequency and distribution of infection in a poultry population as naive populations are exposed to new variants. Vaccination is also thought to exert selection pressure on the virus to increase the mutation rate by several orders of magnitude [10].

In addition, evolution of new clade types can change the morbidity and mortality. In Indonesia and Cambodia prior to 2012, clade 2.2 and clade 1.1 were predominant respectively, with mortality in domestic ducks of less than 10%. With the introduction of clade 3.2.1, duck mortality was reported to be greater than 40% and up to 90% depending on the age of the ducks.

Finally, host-virus interaction is critical in determining the evolutionary polarity and survival of a particular virus subtype and clade in a population, either wild or domesticated. As a virus is transferred from wild birds to poultry, natural selection within the host favours greater adapted strains, which often become more pathogenic for both LPAI and HPAI pathotypes [1].

Host Factors

Under the OIE Terrestrial Animal Health Code poultry "means all domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose" [2].

Transmission may be due to direct contact with oropharyngeal secretions, respiratory aerosols or cloacal content of infected birds. Indirect contact also occurs including fomite transmission (contaminated vehicles, egg cartons, vaccination equipment, etc.) and human movement. Thorough cleaning and disinfection is essential in order to remove all organic material, which may be potentially contaminated.

In the 1960's, LPAI was discovered in wild bird breeding grounds constituting the reservoir for all avian influenza viruses [1]. Once LPAI viruses spill over from wild birds to poultry, specifically LPAI H5 and H7 subtypes demonstrate the potential to mutate into HPAI pathotypes as the virus circulates in poultry population. In 1998, this occurred in Italy over a six-month period however, a review of OIE reports and published studies illustrate that this process can take weeks to years to occur, depending on a number of epidemiological factors. Historically, there have been only two reported instances when an HPAI virus caused mortality in reservoir

hosts: i) in 1961 H5N3 HPAI resulted in sudden death on the South African coast; and ii) Eurasian H5N1 which was first detected in 1996 in Hong Kong (A/goose/ Guangdong/1/1996) (1,6).

Avian influenza virus has been isolated from the following orders of wild avian species: Anseriformes (waterfowl, such as ducks, geese and swans); Charadriiformes, including the Laridae (gulls and terns) and Scolopacidae (shorebirds). Some aquatic species in other orders might also be maintenance hosts. LPAI viruses is not often detected in most wild birds that live on land however, these birds such as passerine and Corbids (crows) can also become infected as reported from India in 2011 (OIE WAHID, 2012 **http://www.oie.int/wahis_2/temp/reports/en_fup_0000011490_20120113_144638.pdf** (Accessed 19 November 2015)).

Swine are important mixing vessels for LPAI viruses in areas when they are located near large poultry populations such as turkeys in Minnesota and North Carolina, USA. However, swine do not play a significant role in relation to the epidemiology of Eurasian H5N1 HPAI [1].

Significantly, waterfowl (e.g. ducks and geese) and water birds (e.g. egrets) can be both high-risk reservoirs and asymptomatic carriers for the introduction, transmission and endemicity of avian influenza virus among various poultry subpopulations [1]. Countries in Southeast and South Asia have proportionately large duck populations and China, the origin of H5N1 virus, A/goose/ Guangdong/1/1996. China possesses 70% of the global waterfowl population [7]. The role of wild birds in the introduction and spread of avian influenza is poorly defined and therefore be considered in the context of specific spatial and temporal risks in each area. FAO recently compiled a summary of key studies supporting or refuting the role of wild birds, presented below.

Waterfowl (domesticated or wild) and gallinaceous poultry may possess cross-immunity if previously exposed to the homologous or a closely related H subtype. Specifically, research has revealed a certain level of cross-protection of H5N1 due to previous exposure with H9N2 [12]. This is biologically plausible due to the commonly shared conserved genes in both virus subtypes, which are widely present in Asian countries.

Table 1: The role of wild birds in past and current avian influenza events [11]

Risk Factor	Reference
Initial introduction of HPAI associated with long distance transmission from infected areas through migratory birds	Si et al., 2009; Newman et al., 2012
Disease persistence and spread associated with poultry population densities, human population	Gilbert and Pfeiffer, 2012

density, land-use patterns, and movement of poultry and humans	
Chicken density	Pfeiffer et al., 2007; Gilbert et al., 2008; Ward et al., 2008; Martin et al., 2011
Duck density	Gilbert et al., 2006
Anthropogenic variables such as human population density have been correlated with HPAI H5N1 risk due to poultry trading and marketing practices	Gilbert et al., 2008; Tiensin et al., 2009; Van Boeckel et al., 2012; Loth et al., 2010; Pfeiffer et al., 2007; Martin et al., 2011; Ward et al., 2008
Road networks are associated with risk of HPAI H5N1 outbreaks	Paul et al., 2009; Yupiana et al., 2010; Biswas et al., 2009; Ward et al., 2008; Martin et al., 2011
Intensification of poultry production may also contribute to increase in HPAI risk	Van Boeckel et al., 2012
Land-cover, such as river networks, presence of inland water bodies are associated with HPAI risk as a direct risk to both poultry and humans; virus can remain infective for several days at ambient water temperature	Nazir et al., 2010; Vong et al., 2009
Spatial and temporal clusters of HPAI have also been identified sometimes associated with rice cultivation	Tiensin et al., 2009; Pfeiffer et al., 2007; Henning et al., 2009; Minh et al., 2009; Ahmed et al., 2010; Loth et al., 2010; Ekong et al., 2012

The species, breed, strain and age of the host are critical demographic factors that determine host susceptibility, morbidity and mortality following virus entry and replication. In general gallinaceous birds (chickens, turkeys, game birds) are more susceptible than waterfowl [1]. Young ducks infected with H5N1 clade 2.3.2.1 experience high morbidity and mortality than older ducks. Quail are effective amplifiers of AI virus and have been implicated in the early outbreaks of H5N1 in Hong Kong. They also possess α2,3 linkage receptors in the respiratory tract epithelium and α2,6 linkage receptors in the gastro-enteric epithelium. Although not definitively proven, quail may potentially amplify spread of LPAI or HPAI with specific receptor binding affinity for humans [1].

Intensive and extensive poultry production systems influence the introduction and transmission of AI virus among the poultry populations. Both commercial and small village based systems are at risk and the Basic Reproductive Number (R_0), or rate of transmission in the population is variable depending on biosecurity risk, contact type and contact frequency. Housing management systems using confined cages versus either free access solid or slatted

flooring systems are also associated with the contact rate and virus transmission rate. Although poultry housed within cages are at high contact, there is relatively limited contact among different penned groups of birds in caged systems. This is in contrast with high contact rates possible in floor housing systems where birds wander freely throughout the poultry house.

The type of production system is critical in order to assess risk of exposure. Epidemiologically, the generation interval of long-lived poultry (egg layers and breeders, heavy weight meat birds) results in a longer duration of exposure to AI virus as compared with short-lived poultry (broilers, light weight meat birds).

Immunosuppressive disease agents of poultry are critical factors to evaluate and include: i) bacteria including E. coli, Pasteurella multocida, Salmonella sp., etc.; ii) aflatoxins from feed; and iii) other viruses such as infectious bursal disease virus, Newcastle disease virus, infectious bronchitis virus, chicken infectious anaemia virus, etc. [1]. The presence of immunosuppressive disease reduces the infectious dose and has been associated with morbidity and mortality due to both LPAI and HPAI co-infection. Therefore, general health screening for concurrent diseases in poultry populations is paramount for preventive (prophylactic) immunization and for general control of avian influenza.

Environmental Factors

Agro-ecological factors (with references) that have thus far been associated with risk for introduction or transmission of H5N1 HPAI among poultry are summarized in Table 2 [11].

The economic, policy and political environment also act as drivers for AI introduction and transmission. These drivers include high demand for poultry in a cross border area, price differentials and non-harmonized culling and compensation policies of neighbouring countries [14][15]. Value chain analysis and social network analyses are therefore important tools, which can be used to characterize the players, power structures and movement patterns within and across national boundaries and the risks associated with these movements [16].

Table 2: Agro-ecological risk factors for H5N1 HPAI from published peer-reviewed references

Risk Factor	Reference
Initial introduction of HPAI associated with long distance transmission from infected areas through migratory birds	Si et al., 2009; Newman et al., 2012
Disease persistence and spread associated with poultry population densities, human population density, land-use patterns, and movement of poultry and humans	Gilbert and Pfeiffer, 2012
Chicken density	Pfeiffer et al., 2007; Gilbert et al., 2008; Ward et al., 2008; Martin et al., 2011
Duck density	Gilbert et al., 2006
Anthropogenic variables such as human population density have been correlated with HPAI H5N1 risk due to poultry trading and marketing practices	Gilbert et al., 2008; Tiensin et al., 2009; Van Boeckel et al., 2012; Loth et al., 2010; Pfeiffer et al., 2007; Martin et al., 2011; Ward et al., 2008
Road networks are associated with risk of HPAI H5N1 outbreaks	Paul et al., 2009; Yupiana et al., 2010; Biswas et al., 2009; Ward et al., 2008; Martin et al., 2011
Intensification of poultry production may also contribute to increase in HPAI risk	Van Boeckel et al., 2012
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Spatial and temporal clusters of HPAI have also been identified sometimes associated with rice cultivation	Tiensin et al., 2009; Pfeiffer et al., 2007; Henning et al., 2009; Minh et al., 2009; Ahmed et al., 2010; Loth et al., 2010; Ekong et al., 2012

Avian influenza viruses remained viable from < 1 day to 7 days at temperatures of 15-35°C (59-95°F) under laboratory conditions. At colder temperatures (4°C; 39°F), virus survival in feces ranged from less than 4 days to at least 30-40 days in different experiments. When protected from sunlight, virus persistence on various surfaces, or in soil, ranged from less than 2 days to more than 2 weeks (and possibly several months), at temperatures ranging from 4°C to 15-30°C (59-86°F). Two studies suggest that virus survival might be particularly prolonged on feathers. In poultry meat (pH 7), a virus survived for 6 months at 4°C. Environmental sampling in Cambodia suggest that avian influenza Eurasian lineage H5N1 virus may survive only several days in tropical environments [17].

Clinical Signs

The HA gene determines the pathogenicity of an AI virus in poultry. The HA cleavability by the host cell at the receptor binding site on the cell membrane is the key interaction producing HA1 and HA2 cleavage by-products permitting cell entry. The hemagglutinin inhibition (HI) test from egg inoculation remains the gold standard test for avian influenza virus [2]. However, clinical signs are in no way pathognomonic for AI, either LPAI or HPAI. Major determinants of clinical signs observed include:

1. The pathotype (LPAI or HPAI);
2. H and N Subtype;
3. Clade;
4. Exposure history;
5. Host characteristics (discussed above);
6. Presence of concurrent (immunosuppressive) disease, including regionally endemic LPAI viruses such as H9N2 documented in Asia and Egypt.

The official incubation period set by the OIE is 21 days [2]. However, the biological incubation period for individual and flock level challenge is considered 3 to 14 days [1]. Clinical signs of avian influenza are highly variable among and within each avian species. Peracute death can occur without exhibiting prior clinical signs associated with avian influenza virus. The following table summarizes an array of clinical signs that may be observed in domesticated gallinaceous birds and waterfowl infected with avian influenza virus [1][17][18].

Waterfowl infected with LPAI viruses are typically asymptomatic, however suppressed T-cell production and depression in egg production has been reported in mallard ducks [1].

Table 3: Clinical signs of LPAI and HPAI viruses in gallinaceous birds and waterfowl

Clinical Signs	Gallinaceous Poultry		Waterfowl	
	LPAI	HPAI	LPAI	HPAI
Depression	+	+++	-/+	-/+++
Decreased intake of water and feed	+	+++		-/+++
Peracute death	-	+/+++		+/+++
Coughing, sneezing, rales, sinusitis, lacrimation (upper airway)	++	+/+++	-/+	+/+++
Oedema of the face, head and neck	-/+	+++	-/+	-/+++
Reduced vocalization (cathedral syndrome)	-	+++	-	NA
Haemorrhage and necrosis of the skin including comb, wattles, leg shanks	-	-/+++	-	NA
Broodiness	-/+++	-	-	-
Drop in egg production	+/++	+++	-/+	NA
Mortality threshold (% if specific estimate given or symbol)	<5% or higher with secondary infection or in young <3 months	Up to 97%	-	-/+++ especially in young <3 months
Diarrhea	-	+/+++	-	+/+++
Nervous signs: Torticollis, opisthotonus, paralysis	-/+	+/+++	-	+/+++
Corneal opacity	-	-	-	-/+++

LPAI only have one single basic amino acid (arginine) at the glycosylation site at amino acid 13 that shields the proteolytic cleavage site. LPAI primarily affects the upper respiratory tract of poultry; typically show mild respiratory signs such as coughing, snicking (sneezing), and lacrimation. Other common signs of LPAI include depression, ruffled feathers, decreased water and feed consumption and possible diarrhea. Egg yolk peritonitis is a common sequelae to respiratory disease, resulting in a drop in egg production and increased broodiness of laying hens and breeders.

Highly pathogenic avian influenza induces a multi-systemic infection due to the presence of multiple basic amino acids located near the HA1 cleavage site, which can be cleaved by ubiquitous furin and trypsin enzymes throughout the body including digestive, nervous and cardiovascular systems. Typical clinical signs of LPAI and HPAI in gallinaceous poultry and waterfowl are summarized in Table 3 [1][18].

Diagnosis

Differential Diagnoses

The following diseases must be considered in the differential diagnosis of virulent AI *causing sudden high mortality* [18]

: Newcastle disease; infectious laryngotracheitis; duck plague/duck virus enteritis; acute poisonings. Other diseases causing swelling of the combs and wattles: acute fowl cholera; and other septicemic and bacterial diseases affecting the comb and wattles. Milder forms of AI may be confused with, or complicated by, many other diseases with respiratory or enteric signs. AI should be suspected in any disease outbreak in poultry that persists despite the application of preventive and therapeutic measures for other diseases.

Gross pathology

The hemagglutinin inhibition test from egg inoculation remains the gold standard test for avian influenza virus [3]. Gross pathological features are highly variable and in no way pathognomonic for either LPAI or HPAI pathotypes. The following list summarizes the gross lesions found [1][18].

LPAI

- **Chickens:** Upper airway catarrhal, fibrinous, serofibrinous, mucopurulent or fibrinopurulent inflammation; tracheal oedema, congestion, +/- haemorrhages with serous and caseous exudate and asphyxiation; infraorbital sinus swelling filled with mucus with mucopurulent discharge; Fibrinopurulent

bronchopneumonia with secondary bacterial infection; “egg yolk” peritonitis with exudate in ovary and oviduct and misshapen eggs.

- **Waterfowl:** Upper airway Sinusitis with discharge; conjunctivitis; air sacculitis**,** fibrinous to fibrinopurulent exudate and inflammation with secondary bacteria.

HPAI

- **Chickens:** Haemorrhage and necrosis of the skin including comb, wattles, leg shanks; subcutaneous oedema and haemorrhages; Severe oedema of face, head, neck haemorrhage and necrosis, particularly Peyer’s patches, pancreas, spleen; Peracute death with no lesions; diffuse interstitial pneumonia, with fluid and haemorrhage; Haemorrhage (petechial/echymotic) in pericardium, pectoral muscle, proventriculus and ventriculus;

- **Waterfowl:** Severe congestion and edema in lung; severe necrotizing tracheitis; congestion and small necrotic foci in spleen; multifocal pancreatic necrosis; congestion and small foci of hepatic necrosis; brain and viscera very congested; corneal opacity; multiple small foci of necrosis with some gliosis in brain; severe tracheitis; multifocal pancreatic necrosis; severe renal congestion.

Diagnostic Tests

The FAO specifies the following protocol for collection and transport of samples to verify the presence of AI virus [19].

Specimens:

Specimens should be collected from birds showing signs of acute disease or recently dead (<24 h). Swabs are taken from the cloacae and oropharynx/trachea of sick and dead birds and then be stored in 3 ml of viral transport medium at low temperature. For dead birds, 3 groups of organs should be separately collected during the necropsy and stored in viral transport medium. These are trachea/lung, brain and digestive organs (pancreas, proventriculus, cecae, intestine). All any obviously abnormal tissue has also to be sampled. Blood should also be collected from live and dead birds (heart blood) for serum testing. Viral transport media can either be prepared locally at a laboratory (it can be isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing antibiotics, for example 100 g/ml gentamicin sulfate, 2 g/ml amphotericin B) or commercial medium may be purchased. Samples should be taken from several birds in the same suspicion flock.

Transport:

Tissues and swab material should be chilled at 4 °C and forwarded on water ice or with frozen gel packs. If delays of greater than 48 hours are expected in transit, these specimens should be frozen at -80 °C and forwarded with dry ice or liquid nitrogen.

International test standards and methods for the diagnosis of AI in avian species are presented below in Table 4 [2].

Table 4: OIE test methods and their purpose for the diagnosis of avian influenza virus.

Table 1. Test methods available for the diagnosis of avian influenza and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification[1]						
Virus isolation	+	+++	+	+++	+	–
Antigen detection	+	+	+	+	+	–
Real-time RT-PCR	++	+++	++	+++	++	–
Detection of immune response[2]						
AGID	+ (Influenza A)	+ (Influenza A)	++ (Influenza A)	+ (convalescent)	++ (Influenza A)	++ (Influenza A)

1 A combination of agent identification methods applied on the same clinical sample is recommended.
2 One of the listed serological tests is sufficient.

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
HI	+++ (H5 or H7)	++ (H5 or H7)	+++ (H5 or H7)	++ (convalescent)	+++ (H5 or H7	+++ (H5 or H7)
ELISA	+	+	++	+ (convalescent)	++	++

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose.
Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
RT-PCR = reverse-transcription polymerase chain reaction; AGID = agar gel immunodiffusion;
HI = haemagglutination inhibition test; ELISA = enzyme-linked immunosorbent assay.

Commercial diagnostic test kits are available but limited. Reagents required to conducts PCR, serology is also limited in many developing countries. Technology for characterization of strains is quite advanced, but sometimes lacking behind in developing countries. Four types of commercial test kits exist including: Antibody detection ELISA, PCR, lateral flow devices and antigens for HI available or they are in development. The Australian Animal Health Laboratory (AAHL) has developed H7N9 primers, probes and HI antigen tests for H7N9 for use is South and Southeast Asia.

The main gaps include the need for cheap, stable and sensitive tests fit for purpose and that are applied in the field according to manufacturer's instructions [20].

Zoonotic disease

Prior to 1997, H5N1, H7N9 and H9N2 have been most commonly associated with human morbidity and mortality. The case fatality rate for H5N1 and H7N9 virus infections in humans is much higher compared to that of seasonal influenza infections. Most H5N1 and H7N9 viruses are resistant to adamantine antiviral drugs, which are not recommended for use.

H5N1

The H5N1 virus subtype, a highly pathogenic AI virus was first reported in humans in 1997 during a poultry outbreak in Hong Kong SAR, China. Since its widespread re-emergence in 2003 and 2004, this avian virus has spread from Asia to Europe and Africa and has remained endemic in poultry in some countries, resulting in the loss of millions of poultry, several hundred human cases, and many human deaths. Outbreaks in poultry have seriously impacted livelihoods, the economy and international trade in affected countries.

The incubation period for H5N1 in humans is longer than for human seasonal influenza ranging from 2 to 8 days and possibly as long as 17 days. Symptoms include high fever, usually with a temperature higher than 38°C, and other influenza-like symptoms (cough or sore throat). Diarrhea, vomiting, abdominal pain, chest pain, and bleeding from the nose and gums have also been reported as early symptoms. The lower respiratory tract is greatly affected.

The primary risk factor for human infection appears to be direct or indirect exposure to infected live or dead poultry or contaminated environments, such as live bird markets. Cooked eggs and meat are safe to eat however slaughter, defeathering, handling carcasses of infected poultry, and preparing poultry for consumption, especially in household settings, are likely to be risk factors.

H7N9

The H7N9 virus subtype, a low pathogenic AI virus, first infected 2 residents of the city of Shanghai and 1 resident of Anhui province, China in March 2013. No cases of H7N9 outside of China have been reported thus far. Containment measures, including the closure of live bird markets for several months, have impacted the agriculture sectors of affected countries and international trade.

The H7N9 virus particularly affects people with underlying medical conditions, especially in older male patients with a history of contact with poultry at live bird markets in urban areas. The primary risk factor for human infection appears to be direct or indirect exposure to infected live or dead poultry or contaminated environments, such as live bird markets. Cooked eggs and meat are safe to eat however slaughter, defeathering, handling carcasses of infected poultry, and preparing poultry for consumption, especially in household settings, are likely to be risk factors.

H9N2

IN 1999 and 2003, H9N2 was detected in two patients with respiratory symptoms in Hong Kong including a 5-year-old boy [22]. Both recovered but it is important to note that H9N2 shares 6 conserved genes with H5N1 and H7N9. H9N2 also results in significant losses to poultry farmers. H9N2 is therefore worthy of further surveillance for both poultry and humans.

Incidence and Prevalence in Selected Countries

Global

Global, regional and country specific data illustrate that avian influenza in avian species is a seasonal, with peak incidence during winter (e.g. Viet Nam) or during rainy season in equatorial countries (e.g. Indonesia). The temporal distribution of avian influenza has declined between 2003 and 2014 as shown in Figure 5 however, Egypt, Indonesia, Viet Nam, Cambodia, China and Bangladesh are still considered to be endemic countries [11].

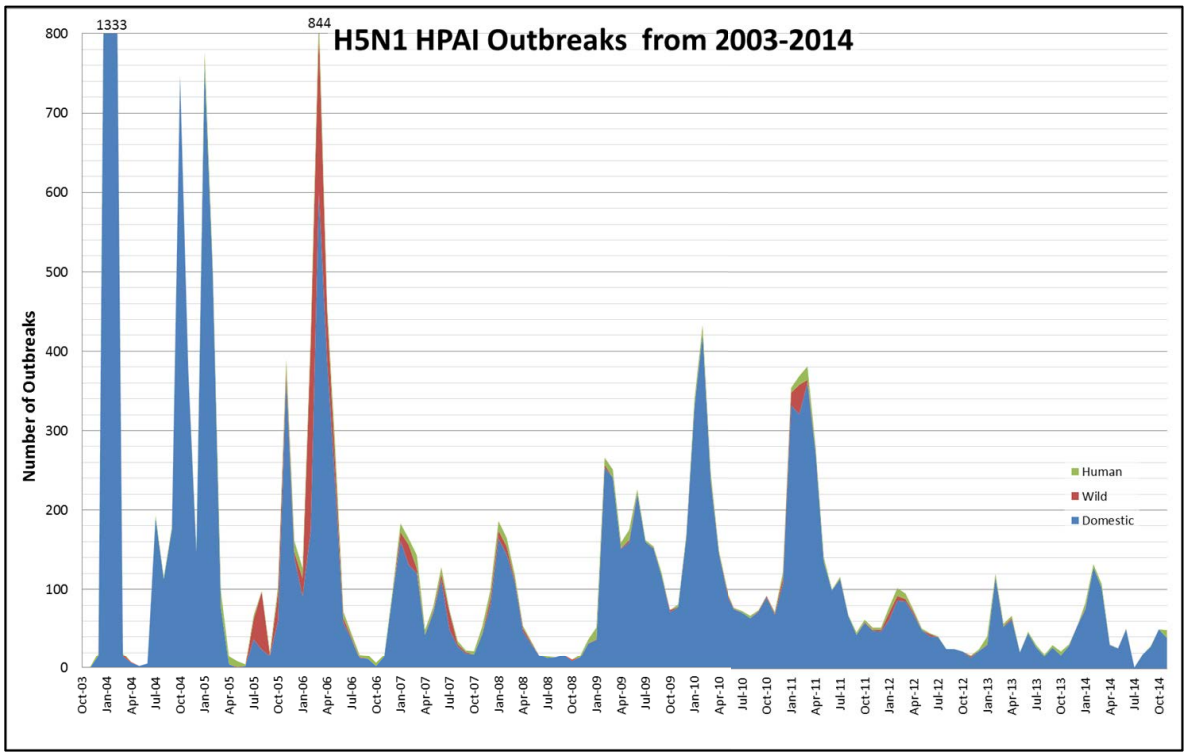

Figure 5: Spatial distribution of NDV events reported in Africa during 2011.

The World Health Organization reports a total 844 human cases and 449 deaths resulting in a case fatality rate of 53% [6].

Of the 20 selected countries of the Livestock Vaccine Innovation Fund, only the following 4 Asian countries have reported human cases and deaths due to H5N1 HPAI (Table 5).

Table 5: Human health impact of H5N1 HPAI in selected, affected countries, 2003-2015

Country	Cumulative Cases	Cumulative Deaths	Case Fatality Rate
Bangladesh	7	1	12.5%
Indonesia	199	167	84%
Myanmar	1	0	0%
Viet Nam	127	64	50.4%

Regional

At least 188 AI disease events were reported from 14 selected African countries and 5,497 AI disease events were reported from 6 selected Asian countries between 2000 and 2015. Regional maps for Africa and Asia are presented in Figures 6 and 7, respectively [23].

Incidence in 20 selected countries

Incidence refers to the number of new cases detected or reported per unit time or per unit of animal-time. For the purpose of this monograph, data is derived from the OIE World Animal Health Information System (WAHIS). CAVEAT: OIE WAHIS data must be interpreted carefully since they have a high degree of uncertainty. The root of this uncertainty can be traced to the country level due to the following reasons:

1. Lack of awareness and capacity to actually detect the disease from local to national levels;
2. Lack of government transparency to report disease when data exists;
3. Rotation of OIE country focal points and non-standardized entry of data due to different interpretation of reporting criteria and coding;

4. Difficulty in defining and applying OIE designated technical terms such as “unit of interest”; “outbreak” and “case” can result in different interpretation in counting disease events.
5. Count data alone (lacking a denominator) must be interpreted with care to avoid over-interpretation.

Methodology

Data from 2005-2015 was collected from WAHIS queries based on the following criteria: 1) Country only for all diseases [24]; and 2) By country and Disease [25][26]. Results from these queries were compared and the higher estimate was entered into Table 6. Data queries for the years 2000-2004 were obtained from an older version of WAHIS termed, HandiStatus II [27]. Incidence data are summarized in Table 6.

Prevalence in 20 selected countries

Prevalence is defined as a count of the number of new and existing disease events reported at, or over a given time period, divided by the total number of samples collected. The denominator permits a more accurate estimate of the burden of disease.

Methodology

Prevalence data (with 95% confidence intervals when available) available for all avian influenza events reported in the 20 selected countries for the years 2000 to 2015 are summarized in Table 7.

Table 6: Avian Influenza incidence data in 20 selected countries, 2000-2015 (OIE, WAHID http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail#) (Accessed 19 October 2015)

Region/Country	Reported Incidence AI															
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Sub Saharan Africa																
Burkina Faso	0	0	0	0	0	…	4	0	0	0	0	0	0	0	0	38
Ethiopia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ivory Coast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Kenya	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Madagascar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malawi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mali	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mozambique	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rwanda	…	…	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Senegal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
South Africa	0	0	0	0	8	0	1	0	0	0	0	50	35*	14*	36*	0
Tanzania	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uganda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Zambia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
South Asia																
Bangladesh	0	0	0	0	0	0	0	68	225	31	30+	169	23	2…	…	…
India	0	0	0	0	0	0	7	1	60	10	5	5	12	3	6	5
Nepal	0	0	0	0	0	0	0	0	0	2	8	1	19	258	1	0
Southeast Asia																
Indonesia	0	0	0	…	…	…	223	…	1161+	1047	…	…	…	462	…	…
Myanmar	0	0	0	0	0	0	78	15	0	0	3	10	2	0	0	4
Vietnam	0	0	0	3	…	1068+	36	73	71	46	44	38	48	39	49*	26

* Includes LPAI

WAHIS Codes 2005-2015

…	No information available for this disease
0	Disease absent
+	Disease present with quantitative data but with an unknown number of outbreaks

HandiStatus II Codes 2000-2004:

0	Disease never reported
…	No information available

Table 7: Avian Influenza prevalence and incidence estimates for 20 selected countries based on peer reviewed literature, 2006-2014 (NA = Not Available)

Region / Country	Apparent Prevalence / Incidence Estimate (95% CI)	Study Design	Time Period	Reference
Sub Saharan Africa				
Burkina Faso	2.4% H5 virus positive	Retrospective survey of active and passive samples from backyard poultry and wild birds	2006	Ducatez, 2007
Ethiopia	NA			
Ivory Coast	0.56% for H5; 0.75% for Influenza A virus	Passive prospective virus survey of wild birds and backyard poultry during active outbreaks	2006	Couacy-Hymann, 2009
	0 (0-04%) and 0 (0-1.48%) in each year	Active Backyard virus survey	2006-2008	Couacy-Hymann, 2012a
	0 (0.04–4.79%)	Active, prospective, live market and backyard flock virus surveillance	2009-2010	Couacy-Hymann, 2012b
	3.65% Influenza A virus prevalence among 1042 chickens	Survey of 44,099 wild birds and backyard poultry from 32 sites in Central and West Africa (incl. Ivory Coast)	2010-2014	Fuller et al, 2015
Kenya	0.8% Influenza A virus prevalence;	Prospective, active cross-sectional study of live markets	2009-2011	Munyua et al, 2012
Madagascar	14% (12-16%) individual seroprevalence for Influenza A	Cross-sectional geo-spatial survey based on poultry seroprevalence	2008-2009	Guerrini et al, 2014
Malawi	NA			

Mali	3.6% virus positive for type A influenza and 13.7% seropositive	Survey of village poultry and live markets in high risk wildlife area	2007	Molia et al, 2010
	AI seroprevalence of commercial farms (0%) and village backyard birds (3.1%) virus prevalence was 1.1%	Prospective, active, cross-sectional survey	2007-2008	Molia et al, 2011
	Very low, year round	Prospective wild bird survey	2008-2010	Capelle et al, 2013
Mozambique	2.51% Influenza A Positive	Wild Bird survey of wetlands in South Africa, Botswana and Mozambique	2007-2009	Cumming et al, 2011
Rwanda	NA			
Senegal	3.5% virus prevalence in wild birds in Africa; the highest prevalence in Mauritania and Senegal; the most frequently infected species were Eurasian and African ducks.	Prospective wild bird survey; cloacal swab samples from captured birds and from freshly killed birds provided by hunters.	2006	Gaidet et al., 2007
South Africa	2.51% Influenza A Positive	Wild Bird survey of wetlands in South Africa, Botswana and Mozambique	2007-2009	Cumming et al, 2011
	6 outbreaks of LPAI in 2014: 306 cases and 159 mortalities; case fatality rate of 51.9%. Cases and deaths due to LPAI in South Africa also showed reduction by 94.2% and 89.1%, respectively from the previous year.		2014	AU-IBAR Year Book, 2014
Tanzania	NA			
Uganda	Influenza A virus prevalence by RT-PCR of 1.1%; seroprevalence (ELISA) of 0.8%	Prospective active cross-sectional survey of live markets	2010-2011	Kirunda et al, 2014

Zambia	0.39% Influenza A virus positive	Prospective wild bird survey	2008-2009	Simulundu et al, 2012
South Asia				
Bangladesh	Attack rates of *upazilas (subdistricts)* of the infected districts: 6/1,000 commercial farms and 1/100,000 backyard flocks	Retrospective study of risk factors	2007	Biswas et al, 2008
	Exact Incidence Rate: 0.0703 per chicken-day at risk;	Retrospective cross-sectional	2007	Biswas et al, 2011;
	23% Influenza A; 0.08% H5 virus positive	Prospective Live market surveillance	2008-2009	Negovetich et al, 2011
	Prevalence of avian influenza type A virus of 22.05%; 39.76% ducks were seropositive AI; Extremely low seroprevalence (0.09%) of AI H5N1.	Prospective, active virus and serosurveillance of duck flocks	2009-2012	Khatun, 2013
India	0.17% (0.14-0.24%) seroprevalence; 0.12% (0.10-0.15%) virus prevalence	Outbreak surveillance (active and passive)	2009	OIE, 2010
Nepal	The incidence of reported AI events due to H5N1 and H9N2 in Nepal increased by 355%	Retrospective spatiotemporal analysis	2010-2012	OIE, 2014; FAO, 2014
	Seroprevalence of AI of 27.2% (24.6-29.5). Of 62 enrolled farms, 42% had at least one seropositive duck. Ducks older than 1 year of age were more likely to be seropositive compared to ducks <6 months of age [odds ratio = 2.17 (1.07-4.39)	Prospective, active, cross-sectional serosurvey of ducks	2011	Karki et al, 2014
Southeast Asia				

Indonesia	H5 bird level seroprevalence was 2.6% for ducks; 0.5% for chickens in contact with ducks; Duck flock–level prevalence of 5.9% to 24.7%	Prospective active multi-stage sampling survey	2007-2008	Henning et al, 2010
	Disease detection rate: 3.8 % (3.7% to 3.9%)	Surveillance risk factor analysis	2006-2007	Loth et al, 2011
Myanmar	NA			
Vietnam	11.74% of 869 communes with significant spatiotemporal clustering for H5N1	Temporal-spatial cluster analysis of communes	2006-2007	Henning, 2009
	10.3% (6-14.5%) seroprevalence in unvaccinated poultry	Cross-sectional survey of poultry in Red River Delta	2008-2008	Desveaux et all, 2011
	12-50% egg yolk antibody test positive for LPAI H3, H6, H8	Prospective live market survey	2010-2011	Hotta et al, 2012
	H5 prevalence (6.6%) from ducks in the Mekong delta	Prospective, cross-sectional active live market survey of ducks	2011	Phan et al, 2013
	Exact incidence rate of influenza type A virus infection was 5 (4-7) positive birds per 100 bird-months at risk	A prospective cohort study of avian influenza infection in poultry flocks was carried out in the Mekong River Delta	2008-2010	Nguyn et al, 2014

Conclusions of AI incidence and prevalence data in 20 selected countries

Burkina Faso and Ivory Coast are at high risk of continuing re-introduction of H5N1 while Indonesia and Viet Nam remain endemically infected. Significantly, Indonesia data are no longer shared with OIE on an ongoing basis due to sensitivities. Conducting surveillance of sentinel chicken flocks near wildlife refuges in Burkina Faso and Ivory Coast would be worthy of consideration due to the low rate of detection in wild birds globally. It is also

important to monitor of poultry traded to these two countries from other neighbouring countries such as Nigeria. There is indirect epidemiological evidence that clade 2.3.2.1 was imported through poultry trade into Indonesia, which highlights the importance of mitigating trade risks. Viet Nam remains a sink for infected poultry from China in addition to re-cycling of virus internally within its borders since both clades 1.1 and 2.3.2.1 are both found in South Viet Nam.

Limited surveillance and reporting is forthcoming from all countries, particularly India. India, Nepal and Bangladesh also remain at high risk as noted through periodic reporting of H5N1. Many countries have relaxed surveillance for H5N1. Note that no countries have reported H7N9 outside of China, despite vigorous surveillance during 2014 and 2015 in the bordering countries including Viet Nam and Myanmar.

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

Global Impact: Very few studies of transboundary animal diseases (TADs) at the regional level exist. The economic impact of the current H5N1 epizootic has been significant, costing tens of millions in 2006 to potentially hundreds of billions of dollars currently at the global level [16][29]. Figure 6 depicts countries most impacted by HPAI, 2006-2010 [28]. Table 8 presents socio-economic impacts due to H5N1 HPAI from peer-reviewed publications.

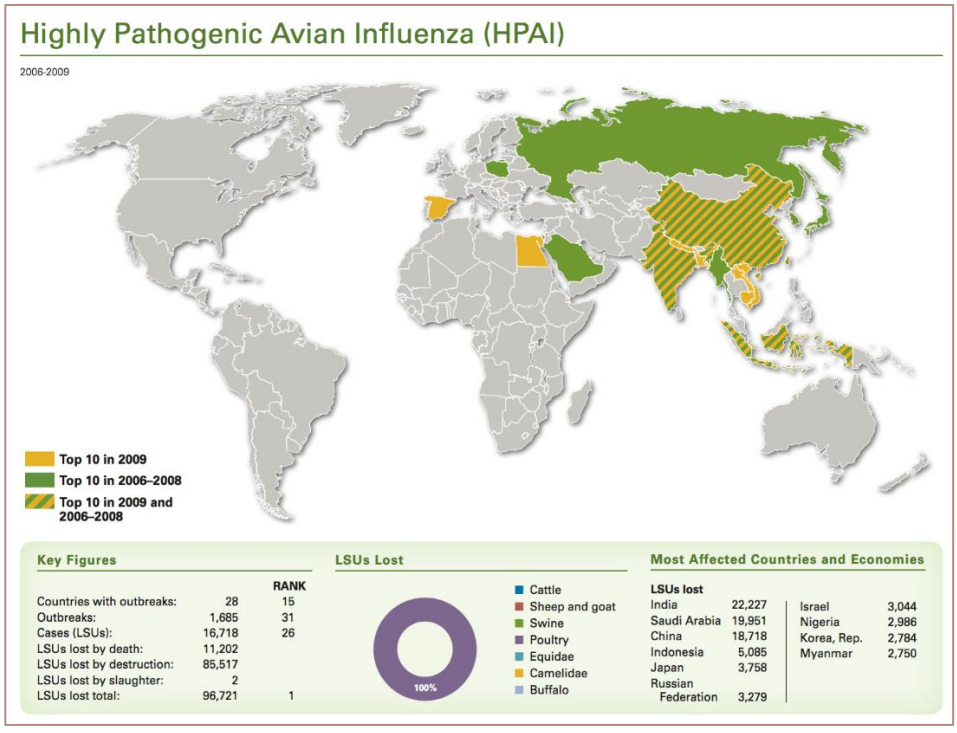

Figure 6: Countries most impacted by HPAI, 2006-2010 [28]

Table 8: Socioeconomic impact of Newcastle disease in 20 selected countries

Region / Country	Economic Impact	Social Impact	Year	Reference
Sub Saharan Africa				
Burkina Faso	NA			
Ethiopia	A livelihoods assessment of the social and economic implications of an outbreak of HPAI was commissioned. The results of this study were used to develop a compensation policy for commercial and backyard poultry production sectors.			Tiongco, 2008
Ivory Coast	NA			
Kenya	Kenya's national preparedness plan includes epidemiology and surveillance, disease control strategies, laboratory diagnosis and research, information, education and communication. The main objectives: 1) strengthen the influenza surveillance network, 2) assess the impact of influenza and benefits of prevention and control		2008	Tiongco, 2008
Madagascar	NA			
Malawi	NA			

Mali	NA		
Mozambique	NA		
Rwanda	NA		
Senegal	NA		
South Africa	NA		
Tanzania	NA		
Uganda	NA		
Zambia	NA		
South Asia			
Bangladesh	February 2007-June 2007: 1.6 million chickens were culled and further 277,000 died in 287 outbreaks; 2.2 million eggs destroyed; 0.8% of poultry lost; 1441 chicks and 733 adult birds were culled per farm, resulting in losses of US$589 and 749 per farm, respectively; Beef and fish prices increased; increased the marketing cost of broilers and eggs by 5 and 14%, respectively	2007-2008	Otte, et al, 2008

India	A typical backyard chicken keeping household that result from culling their chicken flock, one arrives at an estimate of about US$ 13.82 after the inclusion of compensation payments.	With an average flock of 8 chickens, a typical household lost US$ 11.34 culling, of which US$ 5.75 would be offset by compensation. The forgone income would average about US$ 26, raising the total loss per household to US$ 31.6.	2008	FAO, 2008
Nepal	NA			
Southeast Asia				
Indonesia	11 million chickens dead or culled; in 2005, 60% of farms stopped their operations.	More loan requests and less saving in the HPAI-infected farms. Direct impact of HPAI was also seen by decrease in expenditures for education and daily consumption; Levels of social relationship, social networking, social trust, social organization and decision-making remained unchanged.	2003-2009	Basuno et al, 2010
	16.2 million poultry dead or stamped out in control efforts, excluding those lost from backyard farms for which no accurate estimates are available. Estimated loss was US$16.2 to 32.4 million.		2003-2005	McMeod et al, 2007
	July 2003 to 24 January 2004 a total of 15 million layers, 2 million parent stock and 86,000 broilers died or were culled		2003-2004	Rushton et al, 2007

	17.1 million poultry (15 million layers, 2 million parent stock and 0.1 million broilers) died or were culled between July 2003 and January 2004; 6% of poultry population lost; 50-85% price drop for poultry products in January 2004; demand decreased by 58% for broiler Day Old Chicks (DOCs) and by 40% for layer DOCs; prices dropped from US$0.24 (Rupia 2200) to US$0.02 (Rupia 200) per DOC;	A survey of 25 small-scale farms that experienced HPAI H5N1 in Vietnam found that 68% of small-scale commercial farms sold and/or ate dead poultry	2003-2004	Otte, et al, 2008
	Greatest loss was among backyard village farmers, estimated at 30 million households keeping 200 million native chickens or 63% of total poultry population.		2004-2008	Tiongco, 2008
Myanmar	NA			
Vietnam	World Bank macro level estimates for the HPAI outbreaks in Viet Nam of between 0.3% to 1.8% of GDP; $117.5 M total loss in poultry production; average losses per farm affected by HPAI are between US$70 and $108		2004	Rushton et al, 2007
	Overall economic impact may have cost Vietnam between VND 1,500 and 1,800 billion (US$100-120 million), or an estimated 0.3% of GDP		2004	World Bank, 2004

	Loss of 50 million poultry; HPAI-related poultry losses in 2004 represented 25–30% of the total poultry population; Estimated average loss per farm of US$1702 (26.8 million Vietnam Dong (VND); 50-60% price drop for poultry products in October 2005; Prices of non- poultry meats rose by 30% as a consequence of the first epidemic wave in 2004; increased damage from golden snails, increased occurrence of viral diseases in the spring–winter crop in 2006, and as a result lower net incomes	Measures to help farmers cope with liquidity problems were implemented by the Vietnamese government and the Vietnam Bank for Agriculture and Rural Development (VBARD); Reduced number of outlets available to small-scale poultry producers, limiting their commercial opportunities to within-commune trade and some inter-commune trade, thereby also reducing income opportunities for smaller market traders	2004-2006	Otte, et al, 2008
		Employment/income losses for farmers, traders and transporters (many small-scale); % farms (Sector 3 => 38%; Sector 4 => 36%);	2004-2005	Curry, 2006
		Women are heavily impacted: manage small-scale poultry production (CSO; 80% Sector 4); Women involved in trading activities (e.g., 80% traders in Ha Tay, Thai Nguyen Markets)	2004-2006	Curry 2006
	Compensation rate of 30%; The costs of prolonged vaccination in Vietnam are likely to amount to over $39 million		2003-2005	McMeod et al, 2007

Disease Prevention and Control Methods

Prevention and control of avian influenza is deemed a **Global Public Good** due to the serious health and socio-economic impacts of the disease [30].

Treatment (Control)

OIE defines an Official Control Programme as: *"a programme which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country"* [2]. F.A.O. and O.I.E. guidance for the prevention and control of HPAI H5N1 include humane culling of affected stock, movement control, disease surveillance, biosecurity, and vaccination. Biosecurity includes isolation, movement controls, cleaning, and disinfection, as well as disposal of poultry litter and carcasses are essential to support prevention and control. Risk communication, advocacy, public-private partnerships, and compensation are also important requirements for effective implementation of government policies [7].

Prophylaxis (Prevention)

Health interventions apply three strategies for the prevention and control of diseases including preventing exposure, preventing transmission, and enhancing host immunity. Control is part of prevention since controlling the source of virus, prevents further spread. When culling of affected stock, movement control, disease surveillance, biosecurity are not effective enough to prevent endemicity. Vaccination, although costly is therefore a sometimes necessary.

Options and Strategies for Vaccination

Vaccination induces protection to poultry mediated through humoral, cellular, and innate immunity. The immunity that results from vaccination does not provide sterilizing immunity, but it can reduce clinical signs and mortality and reduce virus shedding, resulting in reduced contact transmission among poultry. Inactivated vaccines are most commonly used against H5N1 virus, including traditional and reverse genetics vaccines; however, a number of live-vector vaccines are being increasingly used [29][30]. Key strategic considerations include [7][33]:

1. Vaccine quality, selection, non-replicating and regular matching with circulating field strains;
2. Vaccination strategy – mass/targeted; prophylactic use in breeders/rare breeds; emergency use to protect poultry, farmers and public health; selection of target group;
3. Implementation of vaccination program – public-private partnerships, logistics, procurement, storage, cold chain delivery system, quality control (records)
4. Post-vaccination seromonitoring and evaluation of the vaccination program disease surveillance, and operational research studies to assess impact.
5. Use of multivalent vaccines may be a scientifically valid approach to take when it is possible. The reality in the field is that many economically significant poultry diseases are neither reported nor assessed quantitatively. Vaccine cost as well as farmer and government perception of risk must be carefully assessed in order to prioritize resources for sustainable, self-directed vaccination programs. Needs assessments of the primary stakeholders should first be conducted prior to embarking on prevention and control through vaccination. For example, village poultry owners in Bangladesh indicated to FAO that infectious bursal disease (IBD) is the top priority poultry disease. As a result, biosecurity, training and surveillance systems incorporated specific measures for IBD and AI. The use of NDV-AI vaccines can be considered depending on the results of an epidemiological risk assessment for both diseases.

A questionnaire in tabular format was sent to Directors of Veterinary Services/Directors General/Chief Veterinary Officers on 9 November 2015.

Government Policies and Public/Private Domains for 20 selected Countries are presented in Table 9.

Table 9: Government disease prevention and control policies for the 20 Selected Countries

Avian Influenza (AI)	Notifiable	Official surveillance[1] program	Official control [2] program	Vaccination				Treatment/Chemotherapy	
	(yes/no)	(yes/no)	(yes/no)						
Country				Compulsory vaccination	Who pays for the vaccine?	Who delivers the vaccine?	Species vaccinated	Treatment authorised	Frequently practiced
				(yes/no)	(Government, farmers, combination, others-specify)	(official, private vaccinators or both)	(cattle, sheep, goats, pigs, poultry)	(yes/no)	(yes/no)
Burkina Faso									
Ethiopia									
Ivory Coast	OUI	PASSIF MAIS ACTIF EN CAS D'EPIZOOTIE	OUI	NON				NON	
Kenya	yes	Yes, passive	no	NA	NA	NA	NA	no	no
Madagascar									
Malawi	YES	YES (PASSIVE)	YES	NO	N/A	N/A	N/A	N/A	N/A

Mali	Yes	Yes, passive	Yes						
Mozambique									
Rwanda	Yes	Both	Yes	Yes	Government	Official	Poultry	No	No
Senegal									
South Africa									
Tanzania	yes	Yes, passive/active	yes	no	Not done(disease has not been reported)	Not done	na	no	no
Uganda	YES	NO	NO	NO	NA (never vaccinated)	NA	NA	NA	NA
Zambia									
Bangladesh	Yes	Yes (targeted active)	Yes	Yes	Farmers	Private	Poultry (GPS, PS and Layer)	No	No
India									
Nepal	yes	yes/active	yes	No	N/A	N/A	N/A	N/A	N/A
Indonesia									
Myanmar	yes	yes(active)	yes	no	-	-	-	no	no

Vietnam	Yes	Yes/Active	Yes	No	Government and farmers	Both	Poultry	No	Yes

[1]Surveillance: is the systematic on-going collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

[2]Control: a programme which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country

Vaccines Available

The categories of avian influenza vaccines are presented in Table 10.

Table 10. Categories of avian influenza vaccines manufactured in Africa and Asia

Homologous Inactivated (the same field strain attenuated)
Heterologous Inactivated (a different N antigen based field strain is attenuated)
Reverse Genetics H5 Vaccines (genetic-produced strains based on the hemagglutinin and neuraminidase by altering the HPAI virus hemagglutinin proteolytic cleavage site)
Recombinant Vector Vaccines (in-vivo system; the immunogen is produced within the bird host by use of a live bacterial or viral vector)

Products available in 20 selected countries and doses used are presented in Table 11. Detailed historical information on vaccine production and use is available between 2004 and 2012 [8]: China (120 billion); Indonesia (3 billion); and Viet Nam (2 billion). Inactivated vaccines are most commonly used against H5N1 virus, including traditional, vectored and reverse genetics vaccines; however, a number of live-vector vaccines are being increasingly used [7][30].

Commercial vaccines manufactured in Africa and Asia

Commercial vaccines manufactured in Africa and Asia are presented in Table 11 below. A SWOT analysis for each category of vaccine licensed in the 20 selected countries is presented below [8][35][36][37]. Costs range from $.03-$.05 per dose, administered (2007).

Table 11: List of vaccines manufactured in Africa and Asia.

Specific Vaccines
Homologous Inactivated (the same field strain attenuated)
AVIVAC AI H6N2, Inactivated oil emulsion; DELTAMUNE (Pty) Ltd.; licensed in South Africa
Jova Zeit 1,7 oil emulsion; Jordan Bio-Industries Center (JOVAC); licensed in Ethiopia
Jova Zeit 7; Jordan Bio-Industries Center (JOVAC); licensed in Ethiopia
MEDIVAC AI, Subtype H5N1; Medion Farma Jaya; licensed in Indonesia, Viet Nam
MEDIVAC ND-AI EMULSION Subtype H5N1; Medion Farma Jaya; licensed in Indonesia
Vaksimune AI H5N1 oil emulsion; Vaksindo; licensed in Indonesia, Myanmar
Vaksimune ND AI oil emulsion; Vaksindo; licensed in Indonesia, Myanmar
Antigen AI H5N1 oil emulsion; Pusvetma; licensed in Indonesia
Afluvet H5N1 Clade 2.3.2, Pusvetma; licensed in Indonesia
H5N1 Vaccine Zhaoqing Dahua Agriculture Bio-Pharm Co., Ltd.; licensed in Indonesia and Viet Nam
H9N2 Vaccine Zhaoqing Dahua Agriculture Bio-Pharm Co., Ltd.; licensed in Indonesia and Viet Nam
Heterologous Inactivated (a different N antigen based field strain is attenuated)
*Avian Influenza H5N2; Avimex S.A. de C.V.; licensed in Indonesia, Bangladesh, Nepal
Newcastle influenza H5N2 (also one with hydropericardium syndrome serotype 4; Avimex S.A. de C.V.; licensed in Indonesia, Bangladesh, Nepal
Nobilis Influenza H5N2; Water in oil emulsion; MSD Animal Health; selected countries not listed but was/is used in China (past), Indonesia (past), Viet Nam (past) and Bangladesh (current)

CEVAC® FLU-KEM H5N2 oil emulsion; Ceva Santé Animale; licensed in Burkina Faso, Ethiopia, Ivory Coast, Kenya, Madagascar, Mali, Rwanda, Senegal, Tanzania, Uganda
~CEVAC NEW-FLU KEM® H5N2 oil emulsion; Ceva Santé Animale; licensed in Burkina Faso, Ethiopia, Ivory Coast, Kenya, Madagascar, Mali, Rwanda, Senegal, Tanzania, Uganda
BIO FLU™ H7N1+H5N9 oil emulsion; Merial Italia; licensed in Viet Nam
H5N2 Vaccine Zhaoqing Dahua Agriculture Bio-Pharm Co., Ltd.; licensed in Indonesia and Viet Nam
Reverse genetics H5 vaccines (genetic-produced strains based on the hemagglutinin and neuraminidase by altering the HPAI virus hemagglutinin proteolytic cleavage site)
Bird CLOSE 5.1 oil emulsion; Shigeta Animal Pharmaceuticals Inc.; intended for use in Indonesia
Re-1, Re-3, Re-4, Re-5 monovalent oil adjuvant, reverse genetics; Harbin Veterinary Research Institute, Harbin, Heilongjiang province, China; licensed in Viet Nam and likely unofficially used throughout S. and S.E. Asia
Re-1, Re-3, Re-4, Re-5 monovalent oil adjuvant, reverse genetics; Qingdao Yebio Bioengineering Co. Ltd; licensed in Viet Nam and likely unofficially used throughout South and Southeast Asia
Re-1, Re-3, Re-4, Re-5 monovalent oil adjuvant, reverse genetics; Zhengzhou Bio-pharm Co. Ltd; licensed in Viet Nam and likely unofficially used throughout South and Southeast Asia
^Bivalent H5N1 recombinant, oil adjuvant; Merial, China; licensed in Viet Nam
Recombinant vector vaccines (in-vivo system; the immunogen is produced within the bird host by use of a live bacterial or viral vector)
**Innovac® rND-H5 B1 and H5 H antigens; Avimex S.A. de C.V.; licensed in Nepal, Bangladesh
VECTORMUNE® AI, genetically engineered Marek's Disease vaccine of serotype 3 (turkey Herpesvirus or HVT) expressing Avian Influenza key protective H antigens; HVT serotype 3 is presented in a frozen cell associated form; Ceva Santé Animale; trial use in Bangladesh
Trovac AIV-H5 Pox-vectored recombinant; Merial Select (US); no longer used but licensed in Viet Nam

Commercial vaccines imported into Africa and Asia

Table 12: List of vaccines manufactured in Africa and Asia

Avian Influenza (AI)	Name	Strain or type	Country of origin	Doses imported	Doses imported	Doses imported	Doses imported
				2015	2014	2013	2012
Burkina Faso							
Ethiopia							
Ivory Coast							
Kenya							
Madagascar							
Malawi							
Mali							
Mozambique							
Rwanda							
Senegal							

South Africa							
Tanzania							
Uganda	NA	NA	NA	NA	NA	NA	NA
Zambia							
Bangladesh	Novilis	H5N2	390,500	402,500	-	-	
	Vectorimmune	H5	-	15,709,000	9,524,000	210,000	
	Re-6	H5N1	-	11,436,000	23,936,500	11,769,000	
India							
Nepal							
Indonesia							
Myanmar	No	No	No	No	No	No	No
Vietnam			• Intervet (Nobilis Influenza H5) • Laboratory Avi-Mex SADe CV (Mexico) (K-New 5 – Killed recombinant vaccine against ND and Avian influenza subtype H5) • Boehringer Inhejhem	-	151.823.500	193.238.500	186.850.000

	Vet (H5N2 inactivated) • P.T. Medion • Zoetis • Merial Nanjing Animal Health • Harbin • QYH Biotech Ltd-Lonza Group • Zhaoqing Dahuanong Biology Medicine

Characteristics of Ideal Vaccine Candidates for Smallholders

There are many vaccines for avian influenza subtypes available. Top candidates for Inactivated heterologous monovalent, inactivated combination bivalent, bivalent vector vaccine models are presented below:

Target Product Profile: Inactivated heterologous vaccine model

Attribute	**Minimum** (*Avian Influenza H5N2, Avimex S.A. de C.V.)	Ideal
Antigen	H5 (Heterologous N2); A/Chicken/Mexico/232/94/CPA	Bivalent H5/H9
Indication for use	prevention, control avian influenza subtype H5	same
Recommended species	poultry – chickens, turkeys	Including ducks
Recommended dose	0.5ml; 16% of crude antigen of $10^{8.0}$ CEID50% ml	Maximum – ideally 0.2 ml
Pharmaceutical form	killed AI Type A, subtype H5N2, of chicken embryo origin	same
Route of administration	subcutaneous, neck	mucosal ideally
Regimen – primary vaccination	10 days of age or older	7-10 days
Regimen – booster	2nd in 3 weeks; as needed	every 6 months

Epidemiological relevance and use for smallholders	Potential use in Asia for smallholders	collect baseline data; H5 vaccine for Asia and Africa; H9 also for smallholders in Asia
Recommended age at first vaccination	10 days	7-10 days
Onset of immunity	not specified	4-7 days
Duration of immunity	not specified	4-6 months minimum
Expected efficacy	not specified	> 90% of challenged birds
Expected safety	not specified	100% of challenged birds
Withdrawal period	42 days	28 days
Special requirements for animals	Proper injection away from head - granulomas	Training vaccinators
Special requirements for persons	Tissue reaction from self injection of oil	Effective adjuvant but non-reactive to humans
Package size	500 ml	100 ml
Price to end user	$ 0.03-0.05 US	$ 0.03-0.05 US
Storage condition and shelf-life as packages for sale	Store between 2-7°C (35-45° F). Do not freeze or expose product to direct sunlight.	thermostable
In-use stability	Shake often; Stable for 3 weeks for transportation purposes at 18°C – 24°C (64°F-75°F)	Same

Target Product Profile: Inactivated, bivalent combination vaccine model

Attribute	**Minimum (**~CEVAC NEW-FLU KEM® H5N2; Ceva Santé Animale**)**	Ideal
Antigen	H5N2 A/Chicken/Mexico/232/94/CPA; La Sota NDV	May require B1 or slightly higher pathotype to match field challenge
Indication for use	immunization of susceptible chickens against Newcastle Disease and Avian Influenza type H5	Immunization and protection against NDV and H5 HPAI
Recommended species	chickens	gallinaceous poultry and ducks
Recommended dose	0.5ml	Maximum – ideally 0.2 ml
Pharmaceutical form	La Sota strain of Newcastle Disease virus type A subtype H5N2 Avian Influenza Virus in inactivated form with oil adjuvant; BEI inactivation	May require B1 or slightly higher pathotype to match field challenge;match H5 with field strain
Route of administration	subcutaneous, neck	mucosal ideally
Regimen – primary vaccination	broilers, breeders and laying-type pullets at 8 to 10 days of age.	Same
Regimen – booster	for breeders and laying type pullets, repeat the vaccination at i) 6 to 8 weeks of age; ii) and revaccinate them 3 weeks before the onset of the lay, between 16 to 20 weeks of age.	One booster after the primary, then periodically according to titers
Epidemiological relevance and use for smallholders	potential use in Asia and Africa for smallholders	collect baseline data; also require DVE vectored vaccine for ducks in Asia
Recommended age at first vaccination	8-10 days	7 days ideally

Onset of immunity	not specified	4-7 days
Duration of immunity	not specified	4-6 months minimum
Expected efficacy	not specified	> 90% of challenged birds
Expected safety	not specified	100% of challenged birds
Withdrawal period	not specified	28 days
Special requirements for animals	not specified	No tissue reaction
Special requirements for persons	Tissue reaction from self injection of oil	Effective adjuvant but non-reactive to humans
Package size	500 ml	100 ml
Price to end user	$ 0.03-0.05 US	$ 0.03-0.05 US
Storage condition and shelf-life as packages for sale	Store vaccine between +2°C and +8°C or 35°F and 45°F; protect from light; do not freeze	thermostable
In-use stability	Not specified	Stable before and during use

Target Product Profile: Recombinant vector vaccine model

Attribute	Minimum (**Innovac® rND-H5; Avimex S.A. de C.V.)	Ideal
Antigen	NDV B1 core with LPAI H5 antigen inserted: H5 subtype called VIA Innovac® ® *RND-H5*	match NDV pathotype to local field strains
Indication for use	Immunization of healthy birds	Prevention and control of NDV and AI in poultry
Recommended species	Chicken boilers and breeders	develop non-replicating DVE vector for ducks
Recommended dose	Not specified	
Pharmaceutical form	ENC, B1 strain, which have been inserted genes coding for the hemagglutinin H5 VIA, propagated in SPF chicken embryo and lyophilized	Match NDV strain and AI subtype for epidemiological situation
Route of administration	Ocular (primary), spray, drinking water (boosters)	same
Regimen – primary vaccination	Broilers, the use of the vaccine 1 day of age and older is recommended	Same regimen but also suitable for native "local" breeds
Regimen – booster	Revaccinate broilers between 10 and 28 days old if needed; Layers and breeders: In replacement chicks, two to three applications are recommended vaccine before the onset of lay, with an interval of 3 to 5 weeks apart and every 2 to 3 months as needed.	Community mobilization and ownership required to maintain schedule, including incentives
Epidemiological relevance and use for smallholders	potential use in Africa and Asia for smallholders	collect baseline data; also require DVE vectored vaccine for ducks in Asia

Recommended age at first vaccination	One day old	same
Onset of immunity	not specified	4-7 days
Duration of immunity	not specified	4-6 months minimum
Expected efficacy	not specified	> 90% of challenged birds
Expected safety	completely harmless to birds, even at 10 times the normal dose ocular route and 100 times the normal dose intramuscularly	100% of challenged birds
Withdrawal period	not specified	28 days
Special requirements for animals	once the vaccine used burn, or immerse the container and unused contents in a disinfectant solution	Non replicating
Special requirements for persons	it is safe and harmless to poultry, wild birds, mammals and man	Verify claim
Package size	Blister of 10 vials 1000 doses each	Blister of 10 vials 100 doses each
Price to end user	$ 0.03-0.05 US	$ 0.03-0.05 US
Storage condition and shelf-life as packages for sale	Store in the dark at 2 °C and 7 °C (35 °F 45 °F). Avoid freezing and exposure to direct sunlight.	thermostable
In-use stability	Once the bottle is opened, use the entire contents; do not store bottles with partial content for future use.	Improved quality with smaller dose aliquots of 100

Key Conclusions Related to Vaccination

Short-term Solutions: Inactivated AI vaccines are still effective, produce good titers and can be applied to short-lived poultry. One dose might work for short-lived birds. Proper delivery of vaccine to smallholder with community engagement is a key gap to overcome logistical challenges for the safe and effective delivery of vaccine.

Medium-term Solutions: Some replicating and especially non-replicating recombinant vector vaccines hold promise to minimize barriers to wider and more timely legal licensure, registration and use, particularly for bivalent NDV-AI combinations for chickens and other gallinaceous poultry.

Long-term Solutions: There are two main needs: 1) develop a greater variety of non-replicating vector models, which can be scaled up rapidly, and are environmentally safe; and 2) development of a safe, non-replicating effective bivalent duck virus enteritis-avian influenza (DVE-AI) vaccine for ducks, particularly in Asia.

Limitations

Methodology

This monograph uses an evidence based approach consulting primary referenced studies which summarize key points and considerations for undertaking successful vaccine development. Reporting bias presents a significant handicap for estimating country based risk as explained under section 3. Reporting bias is due to lack of transparency from government officials, lack of incentive to report (low or no compensation paid) and also because farmers themselves ignore or do not report disease. It is common for village poultry owners to eat recently dead or sick poultry due to food scarcity. Community engagement is therefore a critical consideration for any vaccination initiative. As such the prevalence studies are likely of more use, however they must be evaluated and further examined as to the sample sizes and study deigns used, since these greatly affect interpretation of the findings.

Conclusions for short-, medium- and long-term approaches are based on three prevalent or newly developing vaccine models since change will occur during the life of the Project.

Gaps in knowledge or capacity impacting strategic planning and effective implementation

The following gaps are highlighted in relation to vaccine development and sustainable field implementation for avian influenza:

1. The extent of AI vaccine used which does not conform to OIE standards;
2. The need for environmental assessments of replicating recombinant vector vaccines;
3. The need to develop non-replicating recombinant vector vaccines in order to speed up vaccine licensing and registration;
4. Standardized laboratory diagnosis of AI using approved reagents, including primers;
5. Lack of active and passive surveillance systems;
6. Effective programmatic implementation of Differentiating infected from vaccinated animals (DIVA) strategy in more developed and less developed countries remains a challenge. Challenges are related to policy uncertainties as well as laboratory, field epidemiology and operational capacity limitations.

7. China, Viet Nam and Indonesia have ceased to vaccinate smallholder poultry. Sustainable models for successful community engagement to improve logistics and delivery of AI vaccine for smallholders.

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